Laboratory and greenhouse evaluation of a synthetic host volatile attractant for Colorado potato beetle, *Leptinotarsa decemlineata* (Say)

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Abstract

1 The attractiveness of potato plants treated with a synthetic host volatile blend [(*Z*)-3-hexenyl acetate (+/−)-linalool, and methyl salicylate] to newly emerged and 5-day-old adult Colorado potato beetle, *Leptinotarsa decemlineata* (Say), was compared at four doses against untreated control plants and plants treated with an azadirachtin-based antifeedant in greenhouse cage arenas.

2 Attractant-treated plants (derived release rates of 0, 5.7, 17.1 or 57 µg/h) were significantly more attractive than untreated control plants to newly emerged and 5-day-old adults only at 57 µg/h.

3 Attractant-treated plants were significantly more attractive than antifeedant-treated plants to newly emerged and 5-day-old adults at the 5.7 µg/h treatment level and higher. Mean insect density on attractant-treated plants in the attractant/antifeedant study was significantly higher than in the attractant/control study.

4 Habituation to the synthetic attractant was evaluated by exposing adult beetles to the synthetic attractant for 0, 1, 2.5, 4, 8, 12 or 16 h, before release into a wind tunnel in which an attractant-baited plant model was placed at the upwind end. Insects exposed to the synthetic host attractant for ≤ 8 h moved to the synthetic attractant-baited plant model whereas insects exposed to the synthetic host attractant for 12 and 16 h did not. Furthermore, beetles exposed to the synthetic attractant for 0 and 1 h moved at rates greater than, or equal to, the median whereas beetles exposed for longer time periods moved at rates significantly less than the median.

5 These results demonstrate the potential for using the synthetic plant attractant and an antifeedant as components in a stimulo-deterrent strategy for management of the Colorado potato beetle as shown by us in another study.

Keywords Antifeedant, attractant, Colorado potato beetle, plant volatiles, semiochemicals.

Introduction

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is a major pest of crops in the family Solanaceae and is considered to be one of the most destructive insect pests of cultivated potato, *Solanum tuberosum* L., in North America and Europe. This important pest has developed resistance to a wide range of insecticide classes in the U.S.A. (Forgash, 1985; Ioannidis *et al.*, 1991), Canada (Stewart *et al.*, 1997)

and Europe (Forgash, 1985; Boiteau, 1988). Alternative management strategies might reduce this pest's capacity for genetic resistance to conventional chemical controls (Casagrande, 1987) at the same time as providing environmentally sound pest management options to growers.

One alternative to the use of synthetic insecticides is deployment of semiochemicals utilized by insect herbivores in host selection and allelochemicals derived from nonhost plants. Semiochemical-based tactics have the potential to disrupt pest life history without being chemically toxic (Agelopoulos *et al.*, 1999). Several groups have demonstrated that adult *L. decemlineata* are attracted to undamaged potato plants

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(McIndoo, 1926; Shanz, 1953; DeWilde, 1976; Visser & Avé, 1978). In addition, Bolter *et al.* (1997) and Landolt *et al.* (1999) showed that adults are attracted to both artificially damaged and insect damaged potato plants. Dickens (1999, 2000, 2002) identified specific *S. tuberosum* (var. Kennebec) volatiles attractive to both larval and adult *L. decemlineata* and developed a synthetic host volatile attractant blend for this pest.

Azadirachtin is a tetranortriterpenoid allelochemical isolated from seeds of the Indian neem tree, *Azadirachta indica* (A. Juss) (nonhost for *L. decemlineata*). Neem seed extracts have antifeedant activity against over 200 insect species, including *L. decemlineata* (Jacobsen, 1989). Kaethner (1992) reported that the extracts induce morphogenetic defects in *L. decemlineata* larvae, reduce fecundity in adults and lead to feeding inhibition and mortality in both life stages. Zehnder & Warthen (1988) reported that neem application had a negative effect on *L. decemlineata* plant colonization; adult beetles exhibited avoidance or reduced acceptance of neem-treated foliage. One possible pest management strategy is tandem deployment of plant-derived attractants and antifeedants in a stimulo-deterrent program to push pests away from economic crops at the same time as pulling them to sacrificial or noneconomic regions of a production system.

The possibility exists that insect pests habituate to plant attractants and antifeedants and develop resistance that would render these chemicals ineffective for pest management (Schoonhoven, 1977; Jermy, 1983; Jermy *et al.*, 1987). Habituation to both pheromones and antifeedants (Schoonhoven & Jermy, 1977; Jermy, 1983; Jermy *et al.*, 1987; Raffa & Frazier, 1988) has been demonstrated. Electrophysiological evidence (Payne & Dickens, 1976) and behavioural data (Borden, 1967) indicate that sensory adaptation or habituation can occur in pheromone-saturated environments. Synthetic pheromones used for mating disruption can cause sensory adaptation of peripheral receptors or habituation of response at the central nervous system, as was shown for pink bollworm, *Pectinophora gossypiella* (Saunders) (Cardé *et al.*, 1997). Very little work has been carried out to evaluate the potential for insect habituation to plant kairomones. Similar to pheromone-saturated environments, any risk of habituation to plant kairomones might increase in synthetic attractant-saturated environments (Borden, 1967; Payne & Dickens, 1976).

In the present study, our first objective was to assess the attractiveness of plants treated with a synthetic host volatile blend (Dickens, 1999, 2000, 2002) to newly emerged and 5-day-old postdiapause adult *L. decemlineata* against that of untreated control plants and plants treated with a commercially available, azadirachtin-based antifeedant product in greenhouse bioassays.

Our second objective was to determine whether *L. decemlineata* habituate to the synthetic host volatile attractant.

Materials and methods

Greenhouse bioassays

Quantification of volatile release. The attractant blend identified by Dickens (1999, 2000, 2002) was comprised of three-components: (Z)-3-hexenyl acetate (98% purity), obtained from Sigma (St Louis, Missouri); (+/−)-linalool (97% purity), and methyl salicylate (99% purity), both obtained from Aldrich (St Louis, Missouri). The slow release formulation provided by IPM Technologies, Inc. (Portland, Oregon) was comprised of 90% of a proprietary mixture of antioxidants, ultraviolet screening agents, and polymers referred to as LastCall™ (IPM Technologies Inc., Portland, OR). The remaining 10% was made up of the three attractant components: 3.3% (Z)-3-hexenyl acetate; 3.3% (+/−)-linalool; and 3.3% methyl salicylate, by weight.

Release rates for the IPM Technologies, Inc. attractant were determined using an automated volatile collection system (Analytical Research Systems, Inc., Gainesville, Florida) modified from one previously described by Heath & Manukian (1994). These methods are described in detail elsewhere (Dickens *et al.*, 2002). In brief, one 50-μL droplet of synthetic attractant was placed in a 5-L volatile collection chamber, and volatiles were collected over 4 days (two 48-h periods). By programming the switching of eight ports of a manifold holding volatile collection traps containing SuperQ adsorbent (Alltech Associates, Deerfield, IL), it was possible to collect volatiles over 6-h periods for 2 days. After collection, volatiles were extracted from each trap with 100 μL of hexane, of which 50 μL were collected in 300 μL cone vials. N-decane (10 ng) was added to each sample as an internal standard. One microlitre samples were injected into a Hewlett Packard™ Model 5890 A (Hewlett Packard, Palo Alto, California) gas chromatograph (GC) equipped with an HP-5 capillary column (Crosslinked 5% PH ME Siloxane; film thickness 0.25 μm; length 30 m; inner diameter 0.25 mm) and flame ionization detector (FID). The GC was programmed to hold an initial temperature of 50 °C for 2 min after injection, increase 15 °C per min to 235 °C, and hold for 8 min. This experiment was replicated three times. The mean release rate for each component of the attractant was determined for each 6-h period and release rates per hour were approximated. We understand that the derived release rates may not represent amounts actually released by the droplets placed on the plants in the greenhouse environment, and we do not know the effects, if any, of the formulation on volatiles released by the plants during the test period. Thus, the derived hourly release rates are reported only for relative comparisons of the treatments. We were unable to collect volatiles using the same amount of matrix as that used in the wind tunnel assay due to our instrument's high degree of sensitivity to small quantities of volatiles.

Experimental protocol. Adult *L. decemlineata* were obtained from a University of Maine (Orono, Maine) laboratory-reared colony that was established from field-collected beetles in August 2000. Newly emerged and 5-day-old individuals were used to assess potential age-specific differences in attraction to the synthetic host volatile attractant. Experimental work using newly emerged adults was initiated on the day of emergence from the soil ($t = 0$). Adult insects to be tested at 5 days postemergence were maintained on *S. tuberosum* (var. Kennebec) foliage after

soil emergence until experimental work was initiated. All studies were conducted at ambient greenhouse temperature (18–21 °C) and light conditions at the University of Maine entomology greenhouse complex and required 16 days to complete. The Attractant/Control Study ran from 1 February (LD 9.5 : 14.5 h) to 16 February (LD 10.3 : 13.2 h) 2001 whereas the Attractant/Antifeedant Study ran from 20 February (LD 10.5 : 13.5 h) to 7 March (LD 11.4 : 12.6 h) 2001. Due to this close temporal proximity, any within-study changes in environmental conditions, such as temperature and daylight, were judged to have had negligible effects.

Four $1.22 \times 0.61 \times 0.61$ m mesh-enclosed cage arenas were used in each of these studies. Two plastic pots (diameter 20.32 cm), each containing a test plant were positioned 25.4 cm apart within each cage, were laid flush with the cage floor. Field-collected soil concealed both the floor and pot interior to simulate field conditions (Fig. 1). Each pot contained one plant. All study plants were taken from the same 6-week-old cohort, with a mean height of 30.5 cm and mean foliar breadth of 35.5 cm. A thin metal sheet of aluminium flashing (1.52 m $\times 25.4$ cm) placed in elliptical fashion around the floor perimeter precluded insects from accessing the mesh walls, thereby keeping all insects within the arena. All four arenas in a given replicate were located in the centre of the greenhouse, separated by 1 m, and alternated spatially so that two arenas faced east–west whereas the other two arenas faced north–south along the long axis.

The slow-release volatile attractant formulation was applied to potato foliage via plastic cylinders with nozzles calibrated to deliver 50-μL droplets. Upon initiation of each replicate, 0, 1, 3 or 10 50-μL droplets (0, 5.7, 17.1 or 57 μg/h) were applied to the middle third of plant stems to maintain uniform attractant application area for all plants. All four treatments were replicated four times, with each replicate containing all four treatments and one treatment level per arena.

Attractant/control study. The objective of this study was to evaluate the attractiveness of synthetic host volatile attractant-treated plants vs. untreated control plants on the basis of insect choice in cage arenas. Five adult beetles (newly emerged or 5-day-old) were placed in a neutral location on each of the eight cage floors, midway between test plants (Fig. 1). After initiation of the experiment (08.00 h), the location of all insects was recorded at 24, 48 and 72 h. These sampling times were chosen because our primary objective was to evaluate semiochemical effects on insect density over time and not colonization behaviour. Insect location was recorded as attractant-treated plant, untreated control plant, or neutral region, respectively.

Total mean insect density for each age group and insect location within the cage arena was analysed using SAS PROC GLM repeated measures analysis of variance (RMANOVA), with sampling day as the repeated measure and attractant dose as the treatment factor (SAS Institute, Inc., Cary, North Carolina). Modified square root data transformation was conducted to meet condition of normality of $x = (y + 0.5)^{1/2}$. To test for potential effects of sampling day
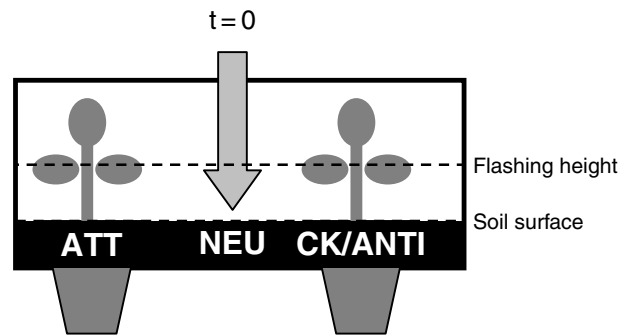

t = 0

ATT   NEU   CK/ANTI

Flashing height

Soil surface

Figure 1 Schematic of cage arena used in greenhouse bioassays. ATT, Attractant-treated; NEU, neutral area; CK/ANTI, control or antifeeedant-treated.

on insect location, degrees of freedom of *F*-tests for within-subject effects were adjusted using the Huynh–Feldt epsilon to account for nonsphericity of the variance-covariance matrices (Huynh & Feldt, 1970). Application of the Huynh–Feldt epsilon (ε) in repeated measures designs adjusts for heterogeneity in both variances and covariances (Winer, 1971; O'Brien & Kaiser, 1985; Keppel, 1991). This procedure corrects for violations in the repeated measures assumption of sphericity by adjusting the degrees of freedom and increasing *P*-values to reduce commission of type II errors. Mean comparisons were conducted using Tukey's *w* Procedure (SAS Institute, Inc.).

Attractant/antifeedant study. The objective of this study was to evaluate the attractiveness of synthetic host volatile attractant-treated plants vs. antifeedant-treated plants on the basis of insect choice in cage arenas. Antifeedant-treatment plants were uniformly sprayed before initiation of the experiment with a 3% azadirachtin product (Azatin® XL; Olympic Horticultural Products, Mainland, Pennsylvania) diluted at a commercial rate of 1.3 mL/L H_2O. Aside from the test dates and the substitution of antifeedant-treated plants for untreated control plants, all other aspects of this experimental design, protocol, and analysis were identical to the Attractant/Control Study.

Wind tunnel studies

Quantification of volatile release. Attractant components, purity and source were the same as in the greenhouse studies. The slow release formulation used in the wind tunnel experiments was provided by Trécé Inc. (Salinas, California) This formulation was a proprietary matrix comprised of 5% by weight of the three attractant components: (Z)-3-hexenyl acetate (+/–)-linalool and methyl salicylate. Release rates of attractant components were determined as previously described for the formulation used in the greenhouse studies with slight modification. In brief, 52 mg of the matrix containing the attractant were placed in a 5-L volatile collection chamber, and volatiles were collected over 3-h periods for 24 h by programming the switching of eight

ports of a manifold holding volatile collection traps; this protocol was replicated two times. The mean release rate for each component of the attractant was determined for each 3-h period and hourly release rates were approximated. The fact that we used a different kairomone formulation for these studies reflects a change in industrial partner. As stated previously, we understand that the derived release rates may not represent amounts actually released by the droplets placed on the plants in the greenhouse environment, and we do not know the effects, if any, of the formulation on volatiles released by the plants during the test period.

Experimental protocol. Habituation to the synthetic attractant was investigated by evaluating the response of adult *L. decemlineata* to the attractant in wind tunnels after laboratory exposure to the kairomone. Adult *L. decemlineata* were obtained from a University of Maine (Orono, Manine) laboratory-reared colony that was initiated by field-collected beetles in August 2003.

Movement of adult *L. decemlineata* toward the synthetic volatile attractant was evaluated in four wind tunnels, each consisting of a 46 cm × 46 cm × 1.8 m long plexiglass box with a blower motor pushing air into the upwind end and a second blower motor pulling air through the downwind end. Airflow was regulated to produce an air speed of 22 cm/s in the tunnel. Before initiation of the experiment, insects were individually exposed to volatiles emanating from 1 mL of the attractant matrix in 5.5-cm diameter Petri dishes for 0, 1, 2.5, 4, 8, 12 or 16 h (LD 16 : 8 h). After exposure, a single insect placed in the centre of the downwind end of the tunnel was allowed to move freely for 1 h. Tunnel position was alternated so that two of the tunnels faced north, whereas the other two tunnels faced south. This experiment was replicated 16 times.

In the centre of the upwind end of tunnel, a 20-cm tall plant model was attached to a 5.5-cm diameter Petri dish containing 1 mL of the attractant matrix. The plant model consisted of green plastic foliage painted with John Deere Yellow[TM] spray paint (Sherwin-Williams Company, Cleveland, Ohio) attached to the top of a 3.0-mm diameter wooden dowel. Attraction of *L. decemlineata* to yellow was previously reported by Zehnder & Speese (1987), Van der Ent & Visser (1991) and Boiteau (2000).

A $\varkappa^2$ test for homogeneity (SPSS Incorporated, Chicago, Illinois) was used to analyse the effects of previous exposure on the proportion of beetles that reached the volatile attractant within 1 h for each pre-exposure time (h). The null hypothesis was that insects exposed to the attractant for different durations before wind tunnel testing were equally responsive to the test attractant. If this hypothesis was rejected, we planned to use the individual treatment chi-square statistics to determine in which treatments insects moved toward the kairomone in significant proportion. A model of these data is represented by nonlinear regression and by a curve fit using SigmaPlot 8.0 (SPSS Incorporated).

A $\varkappa^2$ median test (SPSS Incorporated) was used to assess the effect of duration of previous attractant exposure on the rate at which beetles reached the volatile attractant within 1 h. Movement rates were judged as equal to overall median time and greater than overall median time. The null hypothesis was that insects exposed to the attractant for different durations would move at rates equal to overall median time.

Results

Greenhouse bioassays

Quantification of volatile release. Mean release of each component from a single 50 µL droplet of the synthetic host attractant for each 6-h collection period was: (*Z*)-3-hexenyl acetate, 8.9 ± 0.6 µg; (+/–)-linalool, 15.6 ± 1.6 µg; and methyl salicylate, 8.1 ± 0.7 µg. Thus, under laboratory conditions, the derived release rates for the attractant components were approximately: (*Z*)-3-hexenyl acetate, 1.5 µg/h; (+/–)-linalool, 2.6 µg/h; and methyl salicylate, 1.4 µg/h for a total release rate of 5.5 µg/droplet per hour.

Attractant/control study. There was no significant effect of sampling day on insect distribution among the three regions of the cage arena ($F = 0.19$; d.f. = 6; $P > 0.05$) and no significant insect location by sampling day interaction ($F = 1.74$; d.f. = 6; $P > 0.05$). Insect density data collected at 24, 48 and 72 h were subsequently pooled for analysis. The significance levels of the *F*-tests were adjusted based on a Huynh–Feldt ε of 1.00, as determined by SAS PROC GLM RMANOVA (SAS Institute, Inc.). There were significantly more newly emerged (n = 36) ($F = 118.73$; d.f. = 2, 6; $P < 0.05$; Fig. 2a) and 5-day-old adult beetles (n = 36) ($F = 56.25$; d.f. = 2, 6; $P < 0.05$; Fig. 2b) on attractant-treated plants only when 57 µg/h attractant was released.

Attractant/antifeedant study. There was no significant effect of sampling day on insect distribution among the three regions of the cage arena ($F = 0.29$; d.f. = 6; $P > 0.05$) and no significant insect location by sampling day interaction ($F = 1.67$; d.f. = 6; $P > 0.05$). The significance levels of the F-tests were adjusted based on a Huynh–Feldt ε of 0.85, as determined by SAS PROC GLM RMANOVA (SAS Institute, Inc.). Insect density data collected at 24, 48 and 72 h were subsequently pooled for analysis.

There were significantly greater newly emerged adult mean densities (n = 36) on attractant-treated plants than in the neutral region or on antifeedant-treated plants at nominal release rates of 5.7 ($F = 21.57$; d.f. = 2, 6; $P < 0.05$), 17.1 ($F = 57.49$; d.f. = 2, 6; $P < 0.05$) and 57 µg/h ($F = 78.92$; d.f. = 2, 6; $P < 0.05$) (Fig. 3a). For 17.1 and 57 µg/h rates, insect density on antifeedant-treated plants was significantly greater than in the neutral region. For 5.7 µg/h release, insect density on antifeedant-treated plants was not significantly different from that in the neutral region. For 0 µg/h release, insect densities on attractant-treated and antifeedant-treated plants were not significantly different, whereas both were significantly greater than in the neutral region.

Similarly, there were significantly greater 5-day-old adult mean densities (*n* = 36) on attractant-treated plants than in the neutral region or on antifeedant-treated plants when 5.7
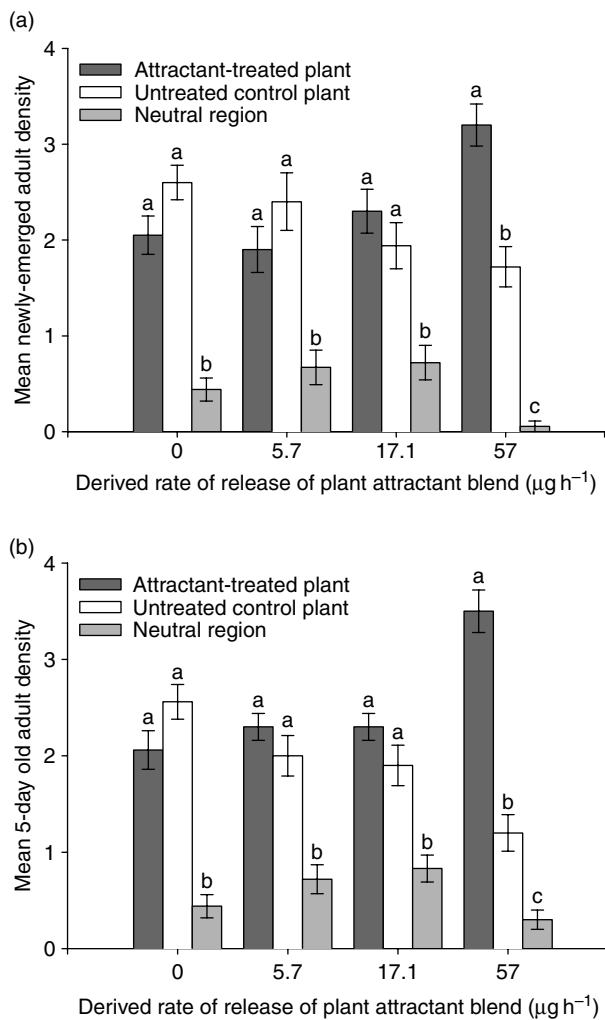
Figure 2 Mean ± SEM density of adult *Leptinotarsa decemlineata* pooled over sampling days on attractant-treated plants, untreated control plants, and in the neutral area of the bioassay arena. (a) Newly emerged adult beetles. (b) 5-day-old adult beetles.

Figure 3 Mean ± SEM density of adult *Leptinotarsa decemlineata* pooled over sampling days on attractant-treated plants, antifeedant-treated plants, and in the neutral area of the bioassay arena. (a) Newly emerged adult beetles. (b) 5-day-old adult beetles.

($F = 26.67$; d.f. $= 2, 6$; $P < 0.05$), 17.1 ($F = 89.94$; d.f. $= 2, 6$; $P < 0.05$) and 57 µg/h ($F = 102.56$; d.f. $= 2, 6$; $P < 0.05$) were released (Fig. 3b). For 57 µg/h release, insect density on antifeedant-treated plants was significantly greater than in the neutral region. For 5.7 and 17.1 µg/h release, insect density on antifeedant-treated plants was not significantly different from the neutral regions. For 0 µg/h release, insect densities on attractant-treated and antifeedant-treated plants were not significantly different, whereas both were significantly greater than in the neutral region.

Wind tunnel studies

Quantification of volatile release. Mean release of each component of the synthetic host attractant from 52 mg of the Trécé attractant for each 3-h collection period ($n = 6$)
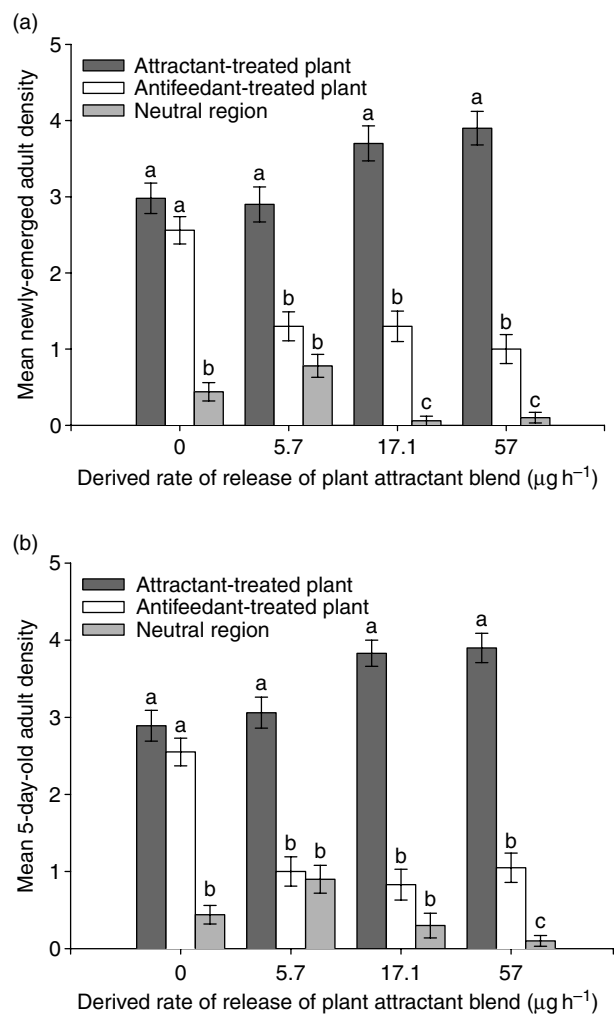
were similar: (*Z*)-3-hexenyl acetate, 9.6 ± 2.0 and 10.5 ± 2.8 µg; (+/−)-linalool, 11.8 ± 2.5 and 11.5 ± 2.3 µg; and methyl salicylate, 6.5 ± 1.0 and 6.4 ± 1.2 µg. Under laboratory conditions, the derived release rates of the attractant components for 1 mL (approximately 1 g) of the matrix used in the wind tunnel experiments were approximately: (*Z*)-3-hexenyl acetate, 65.4 µg/h; (+/−)-linalool, 75.0 µg/h; methyl salicylate, 42.3 µg/h, or approximately 182.7 µg/h total volatile release. It must be noted that because all insects were in dishes only for their indicated pre-exposure time and not all for the same amount of time, the time spent in dishes might have affected their behaviour in addition to duration of odour exposure.

Habituation study. A χ^2 test for homogeneity analysis results determined that the duration of previous exposure to the attractant had a significant effect on the proportion

Table 1 Nonlinear regression of the mean percentage of *Leptinotarsa decemlineata* reaching a synthetic attractant-baited plant model within 1 h by synthetic attractant pre-exposure time

Standard error estimate $= 5.37$	Coefficient	Standard error	t	P
a	90.44	5.91	15.31	0.0001
b	−0.79	0.16	−5.07	0.0071
x_o	3.92	0.19	19.78	< 0.0001

Analysis of variance	d.f.	SS	MS	F	P
Regression	2	7730.75	3865.38	134.18	0.0002
Residual	4	115.23	28.81		
Total	6	7845.98	1307.67		

of insects reaching the wind tunnel attractant within 1 h ($x^2 = 316.1$; d.f. $= 6$; $P < 0.05$). An individual treatment x^2 test for homogeneity analysis determined that significantly higher percentages of insects exposed to the synthetic host attractant for 0 h ($x^2 = 207.8$; d.f. $= 15$; $P < 0.05$), 1 h ($x^2 = 194.3$; d.f. $= 15$; $P < 0.05$), 2.5 h ($x^2 = 217.4$; d.f. $= 15$; $P < 0.05$), 4 h ($x^2 = 231.3$; d.f. $= 15$; $P < 0.05$) and 8 h ($x^2 = 258.7$; d.f. $= 15$; $P < 0.05$) moved to the synthetic attractant-plant model combination. An individual treatment x^2 test for homogeneity analysis determined that the percentages of insects exposed to the synthetic host attractant for 12 h ($x^2 = 1.6$; d.f. $= 15$; $P > 0.05$) and 16 h (no response) did not move significantly toward the synthetic attractant–plant model combination. These data are modelled in Table 1 and Fig. 4.

A x^2 median test analysis of treatment effects on the number of beetles reaching the synthetic attractant at a rate less than or equal to the median rate of beetle movement (0.17 h) determined that the duration of previous exposure affected the rate of beetle movement ($x^2 = 61.8$; d.f. $= 6$; $P < 0.05$). Beetles exposed to the synthetic attrac-

tant for 0 h (0.08 h; $x^2 = 16.3$.; d.f. $= 15$; $P > 0.05$) and 1 h (0.17 h; $x^2 = 7.01$; d.f. $= 15$; $P > 0.05$) moved at rates that were not significantly different from the median rate whereas beetles exposed for 2.5 h (0.27 h; $x^2 = 168.9$; d.f. $= 15$; $P < 0.05$), 4 h (0.55 h; $x^2 = 237.5$; d.f. $= 15$; $P < 0.05$), 8 h (0.63 h; $x^2 = 263.4$; d.f. $= 15$; $P < 0.05$), 12 h (0.98 h; $x^2 = 299.1$; d.f. $= 15$; $P < 0.05$) and 16 h (no response) moved at rates significantly slower than the median rate.

Discussion

Greenhouse bioassays

When presented with a choice between attractant-treated and untreated control plants, both *L. decemlineata* age groups were significantly attracted to the highest treatment level only. When antifeedant-treated plants were substituted for untreated control plants, 10-fold lower treatment levels resulted in a significant attraction response for both age categories. Results of the between-experiment comparison suggest that these differences were due to experimental treatments and not simply a difference in the number of insects leaving the neutral region of the arenas after initiation.

The suggestion made by Zehnder *et al.* (1988) that azadirachtin application negatively affects adult *L. decemlineata* plant colonization by inducing avoidance and reducing acceptance of treated foliage is one possible explanation for the differences that we observed between insect distribution in the two studies. In this scenario, more beetles would have moved to attractant-treated plants at markedly lower attractant treatment levels simply because the antifeedant-treated alternative was unacceptable for colonization. However, this was not the case in our study because mean insect densities on 0 µg/h attractant-treated plants and antifeedant-treated plants were not significantly different. If the antifeedant was effectively reducing the desirability of treated plants, then we would expect that significantly fewer insects would have been observed on antifeedant-treated plants. It was only in the presence of attractant-treated plants that this actually occurred. The low efficacy observed

$$y = \frac{a}{1 + e^{-\left(\frac{x-x0}{b}\right)}}$$

$R^2 = 0.98$
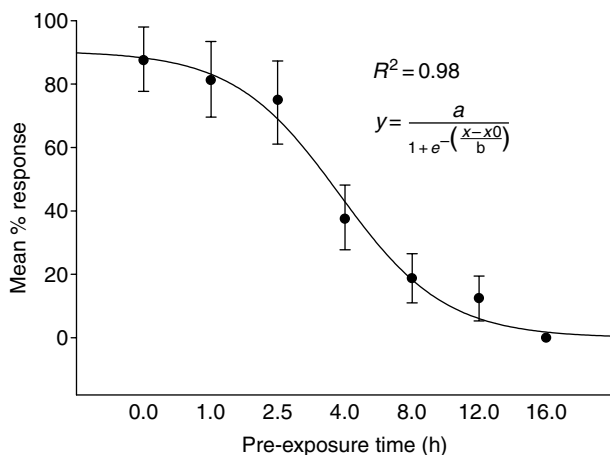
Figure 4 Fitted curve of mean $\pm$ SEM percentage of *Leptinotarsa decemlineata* reaching a synthetic attractant-baited plant model within 1 h by synthetic attractant pre-exposure time.

with regard to adult beetles may be explained by the affirmation that azadirachtin is most effective when small larvae are the predominant life stage (Zehnder *et al.*, 1988).

A more acceptable explanation might lie in the attractant treatment level required to boost host plant desirability above that of alternative options. In the Attractant/Control Study, a level 10-fold less than the lowest treatment level was required to elicit significant attraction for both age categories. In this case, the issue appeared to have been whether or not the attractant treatment levels were high enough to increase host desirability above that of untreated control plants.

Wind tunnel studies

With regard to habituation, our results show that as the duration of pre-exposure to the synthetic attractant increased, successively fewer insects moved toward it and the rate at which they moved decreased sharply. This suggests not only that previous experience affects the onset of habituation, as demonstrated by Raffa & Frazier (1988), but also that the duration of previous experience affects the rate of onset of habituation. Although habituation responses to volatile host plant kairomones are poorly understood, our results suggest that the efficacy of an attractant product may decrease with exposure time to it. Because too much exposure may be counter productive to insect management in the field, more work is necessary to ascertain the threshold below which attractant-treated plants are not sufficiently attractive to *L. decemlineata* and the threshold above which habituation to the attractant becomes an impediment to pest management efforts.

In conclusion, our work has established that potato plants treated with a synthetic host attractant are more attractive than untreated control plants and antifeedant-treated plants to both newly emerged and 5-day-old adults. Pyke *et al.* (1987), Miller & Cowles (1990), and Smart *et al.* (1994) suggested that volatile semiochemicals and antifeedants deployed in tandem in a stimulo-deterrent program might offer growers pest management alternatives to conventional pesticides that rely on insect behaviour modification rather than acute chemical toxicity. In fact, we found that by using a 'stimulo-deterrent' strategy, perimeter treatment with an attracticide in combination with a feeding deterrent was as effective as topical treatment with an imidacloprid insecticide in maintaining potato yield in experimental plots (Martel, Alford and Dickens, unpublished data).

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